PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
G01N 21/64, 21/77

A1

(11) International Publication Number: WO 00/68670

(43) International Publication Date: 16 November 2000 (16.11.00)

(21) International Application Number: PCT/US00/12409

(22) International Filing Date: 8 May 2000 (08.05.00)

(30) Priority Data:
60/133,230 7 May 1999 (07.05.99) US

(71)(72) Applicants and Inventors: ANSLYN, Eric, V. [–/US]; Austin, TX (US). ACANFORA–FLORIN, Sherry, L. [–/–]; – (US). BORICH, Damon [–/US]; Austin, TX (US). DOUGLAS, James, W. [–/US]; Indialantic, FL (US). MCDEVITT, John, T. [–/US]; Austin, TX (US). MCMORRIS, John, A., III [–/US]; Indialantic, FL (US). MULLINS, Gregory, A. [–/–]; – (US). NEIKIRK, Dean, B. [–/US]; Austin, TX (US). OTWORTH, Mike, J. [–/US]; Melbourne, FL (US). SCOTT, John, S. [–/US]; Sebastian, FL (US). SHEAR, Jason, B. [–/US]; Austin, TX (US).

(74) Agents: GARRETT, Arthur, S. et al.; Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P., 1300 I Street, N.W., Washington, DC 20005–3315 (US).

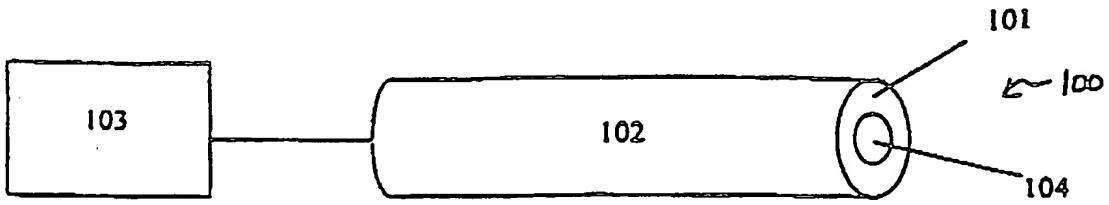
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD AND SYSTEM FOR REMOTELY COLLECTING AND EVALUATING CHEMICAL/BIOCHEMICAL INFORMATION



(57) Abstract

The present invention provides a method and system for detecting analytes in a fluid sample in a system of interest. The method and system includes a sensor, which includes at least one reactant, having an integral detector. The detector detects spectroscopic changes of reactants as the fluid sample passes over the reactants. The spectroscopic changes are caused by an analyte present in the fluid sample interacting with reactants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHOD AND SYSTEM FOR REMOTELY COLLECTING AND EVALUATING CHEMICAL/BIOCHEMICAL INFORMATION

Priority Claim

This application claims the benefit of U.S. Provisional Application No. 60/133,230, entitled Method and System for Remotely Collecting and Evaluating Chemical/Biochemical Information, filed on May 7, 1999.

Background of the Invention

1. Field of the Invention

The present invention relates to a method and device for the detection of analytes. Preferably, the analytes are localized at the end of a probe suitable for insertion into a body of interest. The body of interest may include a wide variety of materials, including, but not limited to, biological systems, both living and dead. Thus, the body of interest may be the interior of an animal (which term is understood to include humans) or a plant. A body of interest could also be a food product, the wash of a food product, as well as agricultural and/or environmental materials.

The present invention allows one to acquire chemical/biochemical assays in localized regions, such as proximate to internal organs or the interior of blood vessels of a human or animal body. If the present invention includes a video monitoring system, the present invention allows one to simultaneously acquire

both optical information related to the condition of the body of interest, such as, for example, the condition of internal organs or blood vessels, while also providing chemical/biochemical assays of these localized regions. More particularly, the present invention relates to the development of instrumentation that may be used for both surgical and microsurgical applications, wherein minimally invasive procedures may be completed with a high degree of accuracy.

2. Brief Description of the Related Art

The development of smart sensors capable of discriminating different analytes, toxins, and bacteria has become increasingly important for a wide range of applications, including environmental, health and safety, remote sensing, food/beverage and chemical processing applications. Sensors have been developed that may detect a single analyte or multiple analytes. Sensors for multiple analyte detection generally include an array of sensors. The advantages of these array sensor systems are their ability to analyze multiple analytes, and their ability to be "trained" to respond to new stimuli through the use of specific receptor molecules and the ongoing development of new types of receptor molecules. The on-site adaptive analysis capabilities afforded by array based sensors displaying the capacity to sense and identify complex vapors have been fashioned using a number of distinct transduction schemes.

If the sensors sample air, as opposed to fluid, the sensors are generally referred to as "electronic noses." Electronic noses generally include a housing for sampling the air sample, which includes the array of sensors, and a system for detecting changes to the sensors of the array. The choice of the type of sensors is dependent on which analyte or how much of an analyte needs to be identified.

Typically, in the housing for sampling the air sample, the air sample is pulled by a vacuum pump through a tube into a small chamber which houses the array of sensors. The tube may be comprised of plastic or stainless steel. In this manner, the air sample contacts the sensor array, causing the sensors to react in particular ways to the air sample. The system for detecting changes to the sensor arrays is then utilized to detect in what ways each sensor reacts to that particular air sample.

In some instances, a washing gas such as an alcohol vapor is applied to the array to remove the air sample from the active surface of the sensors. In re-useable sensor arrays, a reference gas may be utilized to place each sensor to a known state in order to introduce another air sample to the sensors.

Typical electronic noses fall into five categories: conductivity sensors, piezoelectric sensors, metal oxide silicon field effect transistor (MOSFET) sensing devices, optical sensors and spectrometry-based sensing methods.

There are two types of conductivity sensors: metal oxide semiconductors and conducting polymer sensors. Metal oxide semiconductors have been utilized more extensively and are widely available commercially, but conducting polymer sensors are also commonly used and are easier to make than metal oxide semiconductors because they do not need a heating element.

For a metal oxide semiconductor, the metal may be tin, zinc, titanium, tungsten or iridium, one of which is doped with a noble metal catalyst such as platinum or palladium. The doped semiconducting material is deposited between two electrodes over a resistive heating element. The electrodes may be platinum, aluminum or gold, while the substrate in which the resistive heater is placed may be silicon, glass or plastic. The resistive heater is normally a platinum metal trace or wire. The use of tin oxide sensors is described in U.S. Patent No. 5,654,497, issued to Hoffheins et al. These sensors display the capacity to identify and discriminate between a variety of organic vapors by virtue of small site-to-site differences in response characteristics.

For a conducting polymer sensor, the polymer is generally from polymer families of polypyrroles, thiophenes, indoles or furans. The conducting polymer is electropolymerized between two electrodes by cycling the voltage between the electrodes. Because conducting polymer sensors operate at ambient temperature, they do not need a resistive heating element.

For both types of conductivity sensors, a volatile organic compound (VOC) is introduced to the active material (either the metal oxide or the polymer material) and alters the conductivity of the active material. The change in resistance across the electrodes is then measured using conventional electronic circuitry. For metal oxide semiconductors, as a VOC passes over the active material, the resistance between the electrodes changes in proportion to the concentration of the VOC. For conducting polymer sensors, the conductivity of the active polymer material changes in relation to its exposure to the VOC.

Another structure involves a series of conductive polymer layers deposited onto metal contacting layers. When these sensors are exposed to volatile reagents, some of the volatile reagents adsorb onto the surface of the polymer layers, leading to small changes in the electrical resistance of these layers. One can identify and quantify the volatile reagents in the air sample by detecting the small changes in the electrical resistance. This detection process takes only a few seconds, and sensitivities of parts-per-billion may be achieved. This electronic nose system is described in U.S. Patent No. 5,698,089, issued to Lewis et al.

Disadvantages with metal oxide semiconductors include that the baseline response of a semiconductor may drift over a period of time, and that the sensors are susceptible to poisoning by sulfur compounds present in the air

sample. Disadvantages with conducting polymer sensors is the difficulty and time that must be spent to electropolymerize the active material, which may result in variations between batches of sensors. Also, these sensors also tend to drift over a period of time, and they are sensitive to humidity, which may mask the responses to certain odorous VOCs.

The second type of electronic noses are piezoelectric sensors. Piezoelectric sensors may be quartz crystal microbalance devices or surface acoustic wave (SAW) devices. The quartz crystal microbalance devices are comprised of a small resonating disk with metal electrodes on each side of the disk, which are connected to a lead wire. The device resonates at a particular frequency when excited with an oscillating signal. During manufacture, a polymer coating material is applied to the disk to serve as an active sensing material. When an air sample is introduced to the disk, the air sample is absorbed by the polymer coating material, which increases the mass of the device and thereby reduces the resonance frequency. The reduction in the resonance frequency is inversely proportional to the mass of the air sample absorbed by the polymer coating material. The type of air sample to be tested will determine the type of polymer coating material utilized, and there are a large number of polymer coating materials available.

Surface acoustic wave devices are comprised of a piezoelectric substance that has a planar surface. Two metal transducers are placed onto the planer surface, on either side of a polymer coating material. A surface wave travels over the planar surface of the device by applying an AC signal across one of the metal transducers. When the surface wave reaches the second metal transducer, the AC voltage is recreated, although the voltage is shifted in phase as a result of the distance traveled. The phase shift depends, in part, on the mass and absorption properties of the polymer coating material, which are influenced in turn by the gas molecules absorbed.

Surface acoustic wave devices operate at much higher frequencies than quartz crystal microbalance devices, and thus may generate larger changes in frequency. However, surface acoustic wave devices may be less sensitive than quartz crystal microbalance devices because the increased surface to volume ratios means than signal to noise ratios are generally poorer. Disadvantages with both types of piezoelectric sensors are their need for more complex electronics as compared to conductivity sensors.

The third type of electronic noses are MOSFET devices. MOSFET sensing devices generally include a p-type substrate with two n-doped regions with metal contacts labeled source and drain. MOSFETs work on the principle that a volatile organic compound may produce a reaction in a metal when the

compound contacts the metal. The product of the reaction may diffuse through the gate of the MOSFET to change the electrical properties of the device. The sensitivity and selectivity of the MOSFET sensing devices depends on the type and thickness of the metal utilized and operating the particular devices at different temperatures. MOSFET sensing devices have not been commonly utilized in commercial electronic nose systems. However, an advantage to MOSFET sensing devices is that they can be made with IC fabrication processes, thereby reducing batch to batch variations. A disadvantage to MOSFET sensing devices is the need for the reaction products to penetrate the metal layer Thus, the sensing device must have a window to permit gas to interact with the gate structure on the IC chip. Moreover, it is important to maintain a seal for the chip's electrical connections in harsh environments.

The fourth type of electronic noses are optical fiber sensors. Optical fiber sensors are generally comprised of glass fibers with a thin chemically active material coating on the sides or ends of the fibers. The active material responds with a change in color to the presence of volatile organic compounds. A light source comprising one or more portions of the electromagnetic spectrum is utilized to illuminate the active material. The active material contains chemically active fluorescent dyes immobilized in an organic polymer matrix. As volatile organic compounds interact with the active material, the polarity of the dyes is

altered, and the dyes shift their fluorescent emission spectrum. When a pulse of light from the light source interrogates the active material, the fluorescent dye responds by emitting light at a different frequency. A light sensor detects the emitted light and the presence of an analyte may be determined.

Advantages to optical sensors are that they are cheap and easy to fabricate. In addition, it is possible to combine a fluorescent dye to antibody-antigen binding, such that a specific molecule, and only that molecule, may be detected. Disadvantages to optical sensors are the complexity of the instrumentation control system, which makes these sensors cost more to produce, as well as their limited life due to photobleaching.

The fifth type of electronic noses is based on spectrometry-based sensing methods. For one type of spectrometer, a concentrated vapor is injected into the spectrometer, and the spectrometer generates a spectral response which is characteristic of that vapor. In another type, acousto-optic tunable filters are utilized. These filters alter their light-filtering characteristics in response to a change in voltage across their light transmission path. If the voltage is varied, the transmission path of the light through the filter changes, as does the peak wavelength of the light. The result is a time-varying signal that is a function of the light spectrum of the incoming source. A sensor at the output detects a characteristic profile for each odorant being examined.

There are a wide variety of additional electronic nose sensor types whose general operation is known to those practiced in the art. In all cases, however, it should be recognized that a relatively small number of carefully chosen sensors can effect tremendous discrimination between different compounds. Moreover, each of the technologies discussed above lends themselves to miniaturization and, in principle, can be combined with the electronic tongue components discussed in the present application.

In many applications, it is necessary to identify and quantify analytes present in either liquid or solid phase samples. In these applications, the sensor is sometimes referred to as an "electronic tongue." Types of electronic tongues sensors include those which have been referred to as "DNA on a chip," as well as thin films, dyes or beads that act as receptor units, conducting polymers, and pulse spectroscopy cells. A full description of each of these types of electronic tongue sensors is beyond the scope of this invention, although systems based on "DNA on a chip" technology and systems using thin films, dyes or beads that act as receptor units are discussed herein. It is assumed that one of skill in the art is familiar with these types of sensors.

Array sensors based on "DNA on a chip" technology have shown great analytical promise. These devices possess a high density of DNA hybridization sites that are affixed in a two-dimensional pattern on a planar substrate. To

generate nucleotide sequence information, a pattern is created from unknown DNA fragments binding to various hybridization sites. Both radiochemical and optical methods have provided excellent detection limits for analysis of limited quantities of DNA. Proceedings of the National Academy of Science, USA, Stimpson, D.I.; Hoijer, J.V.; Hsieh, W.; Jou, C.; Gardon, J.; Theriault, T.; Gamble, R.; Baldeschwieler, J.D., 92, 6397 (1995). Although quite promising for the detection of DNA fragments, these arrays have the disadvantage of having very little sensitivity to smaller organic molecules. In addition, many of the analytes of interest do not contain DNA components, and would thus not be detectable by this type of sensor. Thus, there is a need for a flexible, non-DNA based sensor.

Optic sensors for evaluating one or more analytes in a fluid sample are known in the art. For example, a system of analyzing fluid samples using an array formed of heterogeneous, semi-selective thin films which function as sensing receptor units is described in U.S. Patent No. 5,512,490, issued to Walt et al. In particular, this patent discloses a support member with an optic array of sensing receptor units positioned on the support member, wherein the sensing receptor units react spectrally different with individual analytes in order to detect analytes of interest.

Similarly, another way to analyze fluid samples is based on using optical fibers to carry a plurality of dyes or beads where the dyes or beads function as

sensing receptor units, such as described in, for example, U.S. Patents Nos. 5,244,636, issued to Walt et al. (dyes) and U.S. Patent No. 6,023,540, issued to Walt et al. (beads) (hereafter "the Walt et al. patents"). Typically, the device includes a single or a plurality of optical fibers. The end of each optical fiber may be etched to form indentations. The beads are placed into these indentations, or the dyes are deposited on the end of the optical fiber. The optical fiber provides illumination to transmit light to the beads or dyes. A detector, such as a charge coupled device (CCD) detector, detects the light emerging from the beads or dyes to determine whether an analyte of interest is present in the fluid sample.

The Walt et al. patents disclose analyzing fluid samples in in vivo or in vitro applications. These patents also disclose utilizing optical fiber strands to provide imaging, as well as performing a chemical analysis of the fluid sample. In particular, because the sensor is made of optical fibers, the sensor permits the direct imaging and viewing of the area in which the sensor is placed. The capability of the sensor to provide imaging and chemical analysis would allow one to use a single sensor in an in vivo application, such as angioplasty, to be able to see where the fiber is traveling to deliver the therapeutic treatment, to use the fiber to deliver the therapeutic treatment, and to concurrently measure the efficacy of the treatment.

One disadvantage of the Walt et al. patents is that they require a sufficiently large optical energy source to adequately transmit the optical signal through the optical fiber. In particular, the Walt et al. patents disclose the use of fluorescent markers to detect, which require high incident excitation energy to cause the fluorescence. The use of fluorescent markers is the primary reason for the requirement of a large energy source. Because of this large energy requirement, the required signal-to-noise ratio to distinguish an appropriate signal increases, thereby making this approach systemically less effective. In addition, the Walt et al. patents disclose small beads or dyes, and thus there is only a small amount of material to react such that the reactant signal is small. Moreover, if optical filters are not utilized to differentiate the return signal from the excitation signal, the signal-to-noise ratio further increases.

Another disadvantage is, because of the use of optical fibers, the sensing device cannot be contained long term within a host body of interest. Instead, because of the use of optical fibers, the fibers and any other needed wires and tubes must come out of the host body to needed equipment that is located outside the host body. For example, the light source and the external image processing device, *i.e.*, the detector or camera, are located at the far or remote end of the optical fibers, outside of the host body. In this manner, the Walt et al. patents require the optical fibers, wires, and tubes to stick out of the host body,

and does not allow for a contained assembly to be inserted into a host body. Furthermore, an additional disadvantage of the optical fibers is that there currently is no mechanism by which one can prevent the beads from becoming dislodged from the optical fiber end.

A method of rapid sample analysis for use in the diagnostic microbiology field is also desirable. The techniques now used for rapid microbiology diagnostics detect an array of various analytes ranging from antibodies, antigens, nucleic acids, enzymes, or metabolites. The most popular of these methods employs antigen-antibody binding. Direct assays involve rapid antigen testing which is based upon the use of antibodies to recognize and bind to either whole organisms, particulate, or byproducts thereof. Inherent to this approach is the need to obtain and characterize the binding of the antibody to unique structures (e.g., epitopes) on the organism, antigen or analyte being tested. This process involves the introduction, identification and isolation of the appropriate antigen-antibody binding pairs and is time consuming. Current diagnostic assays employing these techniques are therefore limited to conducting only a handful of diagnostic assays per testing module in order to avoid interference effects and cross reactions. Moreover, due to the multiple reagent mixing, dispensing, and signaling steps involved in these traditional schemes, there is limited opportunity to conduct simultaneous analysis of multiple antigen-antibody binding events.

The response time of antibody-antigen reactions in traditional diagnostic tests of this type ranges from 10 to 120 minutes, and often involves additional reaction preparation time, depending on the method of detection. The simplest methods are generally agglutination reactions (e.g., blood typing), but these methods are less sensitive and provide limited information due to difficulties in visual interpretation of the reactions. Approaches with slower reaction times include antigen recognition by antibody conjugated to either an enzyme or chromosphere. These test types tend to be more sensitive, especially when spectrophotometric methods are used to determine if an antigen-antibody reaction has occurred.

Second, with respect to diagnostic testing with nucleic acids, one approach uses hybridization to unique regions of the target organism. These techniques require fewer organisms than antigen-antibody binding schemes, but have longer response times, of up to five hours to complete. The most recent improvement in the detection of microorganisms has been the use of nucleic acid amplification. Nucleic acid amplification tests have been developed that generate both qualitative and quantitative data. However, the current limitations of these testing methods are related to delays caused by specimen preparation, amplification, and detection. Currently, the standard assays require about five hours to complete. The ability to complete much faster detection for a variety of

microorganisms would be of tremendous value in a wide range of commercial, medical, and safety applications.

It is therefore desirable that new sensors capable of discriminating different analytes, toxins, metabolic by-products, and/or bacteria in cellular or biological material or in biological exhaust products be developed for medical diagnostic, environmental, health and safety, remote sensing, military, food/beverage, and chemical processing applications. It is further desired that the sensors be adaptable to the simultaneous detection of a variety of analytes to improve throughput during various chemical and biological analytical procedures. In the case of medical diagnostic applications, it is further desirable that the sensors provide real-time or near real-time feedback to facilitate appropriate diagnostic decisions.

The field of medical diagnostics is evolving rapidly, supported primarily by the advancing technological revolution. Aided in part by innovations in the personal electronics and computer markets, devices are constantly being reconfigured and miniaturized and, at the same time, are capable of increasingly intricate functions. This trend is echoed in the field of medicine as patients are demanding similar technological amenities and subsequently raising the accepted standard of care. Several recent innovations, such as the open Magnetic Resonance Imaging (MRI) and "keyhole" Coronary Artery Bypass Graft

(CABG), are evidence of this growing sentiment.

Testing procedures have also been altered in order to accommodate the minimally invasive testing protocols that patients desire. Patients are no longer tolerant of long, laborious diagnostic procedures and shy away from unnecessary sampling or highly invasive procedures. Several emerging technologies, such as electronic chemical/biochemical sensors (including electronic tongues and electronic noses), are especially suited to help meet the needs of this rapidly changing environment. Indeed, these technologies are the epitome of avante garde in the field of medical diagnostics because this equipment may be constructed to be compact, multi-task oriented, minimally invasive, cost efficient, and disposable.

SUMMARY OF THE INVENTION

The present invention is directed to a method of detecting an analyte in a fluid sample. The method includes the step of analyzing the fluid sample integral to a region of interest with a proximally located sensor with an integral detector. The detector may be integral with the sensor by forming the sensor and the detector in a common structure, by attaching the detector to the sensor or through an optical path from the sensor to the detector. The optical path is preferably an optical fiber.

The analyzing step includes the step of providing, for the sensor, a substrate with a plurality of spatially distinct locations. A reactant is associated with at least one of the plurality of spatially distinct locations. The fluid sample is interacted with the reactant. A light source comprising one or more portions of the electromagnetic spectrum is transmitted to the reactant. The detector is then used to monitor the reactant to detect the analyte.

The present invention may include a plurality of reactants. Each reactant may be placed at at least one of the plurality of spatially distinct locations, or more than one reactant may be placed at at least one of the plurality of spatially distinct locations. In the alternative, the substrate defines a plurality of cavities, wherein at least one cavity is placed at at least one of the plurality of spatially distinct locations. Then, each reactant is placed in at least one of the plurality of cavities, or more than one reactant is placed in at least one of the plurality of cavities.

The reactant is selected from the group consisting of a sensing particle, a receptor molecule attached to a sensing particle, a gel or a reactive material. The sensing particle is selected from the group consisting of functionalized polymeric beads, metal oxide particles, thin polymer films, metal quantum particles, semiconductor quantum particles, agarose, ceramic, glass, or any combination of the foregoing. The receptor molecule is selected from the group

consisting of signaling proteins, chemically sensitive dyes, nucleic acid strands, nucleotide sequences, biological binding agents, antibodies, antigens, organic metabolites, inorganic metabolites, functional group receptors, or any combination of the foregoing. The substrate is comprised of glass, plastic, silicon, resin, polymer, fiber composite, metal, metal alloy, ceramic, or any combination of the foregoing.

In one embodiment, the reactant is a fluorescent based reactant, and the light source is selected such that the frequency and wavelength of the light compliments the excitation band of the selected fluorescent based reactant. In an alternative embodiment, the reactant is a colorimetric based reactant, and the light source is a portion of the electromagnetic spectrum. Thus, the light source is a white light.

The detector monitors the reactant as the fluid sample engages the reactant. Spectroscopic changes are caused by the analyte present in the fluid sample interacting with the reactant.

In another embodiment, the present invention is directed to a method of analyzing a fluid sample containing one or more analytes in a biological system. The method includes providing a physical assembly. The physical assembly includes a sensor, a detector and an illumination source. The sensor includes a substrate with a plurality of spatially distinct locations. A reactant is associated

with at least one of the plurality of spatially distinct locations. The physical assembly is introduced proximal to a region of interest where a fluid sample is present. The reactant is interacted with a fluid sample. A light source comprising a portion of the electromagnetic spectrum is transmitted to the reactant. The detector is used to monitor the reactant to detect an analyte while the detector is proximally located to the region where the fluid sample is present.

In a preferred embodiment, the method includes means for retrieving optical information from within the biological system. Preferably, the means for retrieving optical information includes a video monitor.

In a preferred embodiment, the method includes drive and interface electronics connected to the physical assembly, which may be directly or indirectly connected to the physical assembly. The physical assembly may be a probe, or may be spheroidal in shape. The detector is used to monitor the reactant as the fluid sample engages the reactant. The spectroscopic changes are caused by the analyte present in the fluid sample interacting with the reactant.

In another embodiment, the present invention is directed to a method of detecting an analyte in a fluid sample within a biological system including analyzing the fluid sample while inside the biological system with a sensor and a detector which is integral with the sensor. The sensor and the integral detector

are inserted into the biological system to be proximally located to the fluid sample in a region of interest. The detector detects the analyte present in the fluid sample while the detector is proximally located to the fluid sample in the region of interest.

The biological system may be a mammalian body, such as a human, either living or dead. In one embodiment, the sensor and the detector are integrated with a catheter to introduce the sensor and the detector to the region of interest.

The present invention is also directed to a device for detecting an analyte in a fluid sample integral to a region of interest. The device includes a physical assembly. The physical assembly includes a sensor, a detector which is integral to the sensor, and an illumination source. The sensor is comprised of a substrate having a plurality of spatially distinct locations, at least one of the spatially distinct locations having a reactant. The physical assembly introduces the detector proximate to a fluid sample integral to a region of interest to allow the detector to monitor the reactant while proximally located to the fluid sample.

The detector may be integral with the sensor by forming the sensor and the detector in a common structure, by attaching the detector to the sensor, or through an optical path from the sensor to the detector. Preferably, the optical path is an optical fiber.

The device preferably includes a plurality of reactants, wherein each

reactant is placed at at least one of the plurality of spatially distinct locations or more than one reactant is placed at at least one of the plurality of spatially distinct locations. In one embodiment, the substrate defines a plurality of cavities, wherein at least one cavity is placed at at least one of the plurality of spatially distinct locations. Thus, each reactant is placed in at least one of the plurality of cavities, or more than one reactant is placed in at least one of the plurality of cavities.

The reactant is selected from the group consisting of a sensing particle, a receptor molecule attached to a sensing particle, a gel or a reactive material. The sensing particle is selected from the group consisting of functionalized polymeric beads, metal oxide particles, thin polymer films, metal quantum particles, semiconductor quantum particles, agarose, ceramic, glass, or any combination of the foregoing. The receptor molecule is selected from the group consisting of signaling proteins, chemically sensitive dyes, nucleic acid strands, nucleotide sequences, biological binding agents, antibodies, antigens, organic metabolites, inorganic metabolites, functional group receptors, or any combination of the foregoing. The substrate is comprised of glass, plastic, silicon, resin, polymer, fiber composite, metal, metal alloy, ceramic, or any combination of the foregoing.

In one embodiment, the reactant is a fluorescent based reactant, and the light source is selected such that the frequency and wavelength of the light

compliments the excitation band of the selected fluorescent based reactant. In an alternative embodiment, the reactant is a colorimetric based reactant, and the light source is a portion of the electromagnetic spectrum. Thus, the light source is a white light.

In a preferred embodiment, the device includes means for retrieving optical information from within the region of interest, which is preferably a video monitor.

The physical assembly may be a probe, or may be spheroidal in shape. In one embodiment, the sensor and the detector are integrated with a catheter to introduce the sensor and the detector to region of interest.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of one embodiment of the present invention that analyzes a gaseous fluid sample utilizing a video pick-up assembly, and utilizing drive and interface electronics that are indirectly connected to the physical assembly;

Figure 2 is a perspective view of one embodiment of the present invention that analyzes a gaseous fluid sample utilizing a video pick-up assembly, and utilizing drive and interface electronics that are directly connected to the physical assembly;

Figure 3 is a perspective view of one embodiment of the present invention that analyzes a liquid fluid sample utilizing a video pick-up assembly, and utilizing drive and interface electronics that are indirectly connected to the physical assembly;

Figure 4 is a perspective view of one embodiment of the present invention that analyzes a liquid fluid sample utilizing a video pick-up assembly, and utilizing drive and interface electronics that are directly connected to the physical assembly;

Figure 5 is perspective view of one embodiment of the sensor of the present invention; and

Figure 6 is a side view of the sensor shown in Figure 5, with a detector and

illumination source.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the present preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings.

The present invention is directed to method and system for analysis of a fluid that contains one or more analytes in a system of interest. A system of interest may include a wide variety of materials. These materials include all types of biological systems including, but not limited to, medical, animal, environmental and agricultural applications. Thus, the body of interest may be an animal (e.g., mammals, such as humans, dogs and cows, and other animals such as fish) or a plant (e.g., plants, trees, shrubs and the like). In addition, the body of interest could be a living or deceased animal or plant, and thus includes, but not limited to, living or deceased animals, mammals, humans, plants, trees and the like. The body of interest could also be a food product, or the wash of a food product.

The present invention may be used for either gaseous fluids or liquid fluids. Thus, the present invention may utilize either "electronic nose" or "electronic tongue" technology, as well as combining features of both technologies. With respect to sampling gas samples, the present invention may utilize any type of

electronic nose sensor, including, but not limited to, conductivity sensors, piezoelectric sensors, metal oxide silicon field effect transistor (MOSFET) sensing devices, optical sensors and spectrometry-based sensing methods. In addition, with respect to sampling liquid samples, the present invention may utilize any type of electronic tongue sensor, including, but not limited to, those based on "DNA on a chip" technology; those based on thin films, dyes or beads that act as receptor units; probe arrays; conducting polymer gas sensors; acoustic wave gas sensors; field-effect gas sensors; electrochemical gas sensors; pellistors; fiber optic gas sensors; discotic liquid crystals; and pulse spectroscopy cells. Again, the present invention is configured to allow one to combine a type of electronic tongue sensor with a type of an electronic nose sensor to allow one to sample both liquids and gases simultaneously.

Depending on the type of sensor utilized, the detector includes, but is not limited to, optical detectors, such as, for example, complementary metal oxide semiconductor (CMOS) detectors, photovoltaic cells, amorphous silicon photo-sensor on glass (e.g., photodiode or TFT based), Organic Light Emitting Diode (OLED) detectors, infrared detectors, fluorescent detectors, ultraviolet detectors or charge coupled device (CCD) detectors.

The device of the present invention is suitably configured such that the detector is integral with the sensor in a common structure. In this manner, the

actual detection of analytes takes place while the sensor and detector are proximally located to the fluid sample, when the fluid sample is integral with its system or body of interest. There are a variety of manners in which the sensor and detector may be integral with each other. For example, the sensor and detector may be formed in a common structure, such as, for example, in the same mold or die. In addition, the detector may be attached to the sensor through any number of attachment mechanisms, including, but not limited to, the use of adhesives or fastening devices. Also, the detector may be situated near the sensor, but not be part of the same mold of the sensor nor be attached to the sensor. In this situation, an optical light path from the sensor could be transmitted to the detector from the sensor through a variety of mechanisms, including, but not limited to, an optical fiber. In one embodiment, the detector is situated below the sensor such that light from the sensor is transmitted to the detector.

The method and system of the present invention includes a sensor which is preferably comprised of a substrate having a plurality of spatially distinct locations, at least one of the spatially distinct locations having a reactant. The substrate is any material capable of having a reactant incorporated therewith. There are a variety of manners in which the reactant may be incorporated with the substrate. In one embodiment, the reactant is attached to the substrate. In another embodiment, the reactant is intercalated within the substrate. Preferably,

there is a plurality of reactants, each reactant being placed at one of the spatially distinct locations. In the alternative, more than one reactant may be placed at one of the plurality of spatially distinct locations. In a preferred embodiment, the substrate defines a plurality of cavities, with at least one cavity being positioned at at least one of the spatially distinct locations. Thus, in this embodiment, one reactant may be placed in at least one of the cavities or, in the alternative, more than one reactant may be placed in one of the cavities. The spatially distinct locations are comprised of locations spaced apart from one another on the substrate. These locations may be in an ordered array or may be unordered.

The reactant is any material capable of interacting with an analyte that experiences changes that are detectable and that are indicative of the analyte present. The reactant may be a sensing particle, a receptor molecule attached to a sensing particle, a gel, or a reactive coating.

The method of analyzing a fluid sample for an analyte of interest includes having a sensor preferably comprised of a substrate having a plurality of spatially distinct locations, wherein a reactant is associated with at least one of the plurality of spatially distinct locations. To detect the analyte, the fluid sample is interacted with the reactant, and a light source comprising a portion of the electromagnetic spectrum is transmitted to the reactant. Because of the interaction between the analyte and the reactant, the reactant will absorb electromagnetic radiation at one

or more frequencies. The detector is used to monitor the reactant to sense changes in absorbed electromagnetic radiation, and thus spectroscopically detect the analyte.

Uses of the Present Invention

The present invention may be utilized in a wide variety of applications to perform localized assays in specific regions of interest. In particular, the present invention is configured to be able to be placed in the specific region of interest, and is able to perform detection of analytes in a sample at the region of interest while proximally spaced in the region of interest.

For human and animal applications, the present invention may be utilized to determine a variety of local biochemical or chemical conditions that can be used to indicate or diagnose specific local or systemic disorders or states. For example, the present invention may be used to isolate the presence of specific biochemical "markers," such as cardiac or tumor markers, contained with biological serum or at a tissue junction. As such, it may prove useful in researching causal or predictive links between specific markers and known health states, such as unstable angina. As another example, the present invention may also prove useful for discriminating between different internal deposits, such as, for example, lipid laden plaque versus other plaques inside cardiac arterial walls,

thereby providing clinicians with diagnostic data that can help determine patient health states.

The present invention will be capable of performing a multitude of varying forms of analyses and is particularly well suited for minimally invasive diagnostics protocols requiring real-time site-directed feedback data. Unlike existing diagnostic equipment, the present invention requires minimal sample volume and processing, and thus is able to operate in what previously were inaccessible or inoperable mediums. Miniaturized platform size and a high degree of integration allow placement of the system into several existing medical applications. A variety of minimally invasive diagnostic protocols involving catheter placement exist currently that allow real-time monitoring of parameters, such as, for example, intra-arterial pressures and pH. However, no technology currently exists that allows an extensive panel of real-time analysis to be conducted concurrent with minimally invasive procedures.

There are three main areas where the present invention would provide great improvement in current medical diagnostic capabilities. The first involves continuous monitoring of critical care (ICU) patients. These patients, generally suffering in some aspect from a deregulation of homeostasis, often need to have simple blood chemistries, such as, for example, electrolytes and blood gases, drawn on a rigorous hourly basis. For example, the treatment for a patient

admitted in Diabetic Keto-Acidosis consists almost entirely of correcting fluid electrolytes while stabilizing blood glucose levels. This is often an arduous task that, in some circumstances, amounts to "chasing your tail" as corrections in treatment are dependent upon the results of the hourly blood draws. The present invention would provide one the ability to monitor, in real-time, electrolytes, blood glucose, ketones and blood gas parameters, and thus would enable the physician to more efficiently tailor the patient's regimen. Optionally, this could be accomplished through the integration of the chemical/biochemical sensor into a typical catheter that is used to provide central venous access for hydration.

Several other examples exist in which real-time monitoring would provide a distinct advantage in the acute care setting. Poly-trauma patients, often afflicted with similar hemostatic challenges, could also benefit greatly from such diagnostic integration. Moreover, patients admitted for suspected bacterial sepsis could be monitored for the development of complications such as Disseminated Intravascular Coagulopathy (DIC), a commonly fatal state. In this situation, the present invention could monitor parameters associated with developing a Coagulopathy, such as fibrinogen and fibrin split products, could simultaneously help characterize the offending bacterial agent, and thus help determine the appropriate anti-microbial therapy.

The second area in which the present invention would provide great

improvement in current medical diagnostic capabilities is with its integration with new generation endoscopic and laparoscopic technologies. There are many new technologies, both therapeutic and diagnostic, that involve minimally invasive procedures. On a daily basis, endoscopes are utilized in the field of medicine to survey a variety of things such as, for example, gastric ulcers, tumors, colon polyps, and tissue abnormalities. However, little information other than visual cues is provided to the physician to make their clinical assessment. Quite often, the physician is put in the position of taking a conservative approach and either removing or taking a biopsy of a potential malignant or atypical appearing entity. Many of these procedures, as is learned from subsequent pathology reports, are unnecessary and could have been avoided had the proper information been provided. For example, if a physician performing an endoscopic procedure to survey a patient's gastric ulcer was able to detect the presence of the bacteria Helicobacter pylori, he or she may be able to avoid an unnecessary biopsy for possible malignancy by detecting this ulcer causing bacteria.

Similar guidance of therapeutic decisions could be provided in a concurrent fashion in the use of bronchoscopy. Frequently, bronchoscopy is utilized to sample inaccessible fluids or organisms present in the lungs. By using the present invention, a physician would be able to both visualize an area of interest and simultaneously sample the local environment looking for specific bacterial

metabolites or fluid characterization.

Comparable uses for this technology may also be seen in the use of colonoscopy. Colonoscopy is characteristically used to survey anatomical aberrations such as, for example, neoplasms and polyps and local area of inflammation in the intestinal tract. Through the incorporation of tumor marker agents such as carcino-embryonic antigen (CEA) for colon cancer and local cytokines for indicators of specific inflammatory diseases such as, for example, Crohns or Ulcerative Colitis, the clinician may be able to enhance his or her diagnostic ability and survey more intensely for specific etiologic agents of disease.

The third area in which the present invention would provide great improvement in current medical diagnostics is in surgical intervention and bioengineering. The field of surgery, predicated upon its distinct ability to be invasive yet beneficial, has perhaps made the greatest leap to the realm of minimally invasive diagnostic and therapeutic techniques. With the advent of the laparoscope and the accompanying surgical tools, surgeons are now able to perform a majority of their procedures through minute incisions. Although this is obviously a desirable shift for the patient, it has provided a technical challenge and has limited, in some aspects, the surgeon's ability to completely visualize the procedure.

Intricate fiberoptic laparoscope with illumination sources linked to viewing monitors are commonplace in every operating room. However, there are still inaccessible regions that were previously accessible in the more open invasive approaches. The present invention could provide information regarding the viability of tissues by sensing necrosis, thrombosis, decreased perfusion, and local inflammation and local tissue metabolites. With this information, a surgeon would be able to custom tailor his or her procedure around specific biochemical thresholds indicative of disease or anatomical aberration.

For example, a patient undergoing an aortic arch vascular repair often has blood flow diverted from organs such as his or her kidney and colon. Continuous monitoring of local metabolites indicative of stress such as, for example, lactic acid or cellular breakdown components, would provide the surgeon with specific information regarding the viability of bypassed organs. In the case of open heart surgery, a real-time intra-cardiac monitoring of enzymes associated with myocardial infarction, such as, for example, CK-MB, would help eliminate "silent heart attacks" that occur during such procedures. This type of technology would not only give the added advantage of providing real-time data feedback but would also allow specific localized surveys of anatomical regions to be conducted, and thus serve as an extension of the surgeon's diagnostic acumen.

In addition to medicine, the present invention may be utilized in food safety

applications. In particular, the present invention could be utilized to detect pesticides, hormones, toxins, antibiotics, bacterial contamination, and levels of lipid, sugar and oil. Recently, studies have shown an alarming incident of pesticides and hormones in produce purchased in supermarkets. Following up on a 1993 National Academy of Sciences study critiquing federal pesticides standards relating to protection of infants, the Environmental Working Group (EWG) commissioned laboratory tests of baby food made by industry leaders. It found 16 pesticides, including three carcinogens, five 'possible' carcinogens, eight neurotoxins, five endocrine disruptors, and five 'toxicity one' chemicals.

Because of the widespread industry of use of recombinant bovine growth hormone (rBGH) to boost milk production, most dairy cows suffer from clinical mastitis, a common infection of the udder. The antibiotics used to treat these cows often finds its way into our milk. The present invention may be used to sample a wash from fruits, grains or other produce, or to directly sample a solution made from these. Alternatively, the present invention may be used to test liquid food products, such as milk, for example, to monitor for pesticides, bacterial presence, hormones, antibiotics, rodent feces, or specific fungal toxins, such as afaltoxin. Still further, the present invention may be used in ruminant protein screening, for example, to screen for bovine albumin and ovine albumin, which could help document compliance to prevent Bovine Spongiform

Encephalopathy (BSE), also known as "mad cow" disease, as well as to detect other residues.

There is also growing concern regarding the accelerating use of genetically modified crops and their purity. There is evidence that in certain cases genetically modified crops actually concentrate toxins, or in some cases, express proteins whose metabolic breakdown products are toxic to the person or animal that consumes the crop. The present invention could be used to monitor for specific toxins, proteins, and even for specifically coded DNA sequences to ensure crop safety.

Food safety is an ever present concern in meat processing operations as well. Bacterial contamination is a significant health risk that is often caught after meat processing and distribution has already occurred. Because of its unique capability to detect specific bacteria, the present invention could innovate the meat processing industry by providing immediate feedback regarding the presence of specific bacterial contamination, either directly at the surface of the meat or in meat wash products. As well, the present invention could be used to monitor for a wide range of residues, including, but not limited to, toxins, pesticides, hormones, antibiotics, specific fungal toxins (such as aflatoxin), and the like.

Food grading is another area where the present invention would be of use.

This system can be used in meats to detect lipid levels and other factors that correlate directly with "eatability." In crops, the same techniques can be used to detect factors such as, for example, specific oil and sugar levels.

Embodiments of the Present Invention

The present invention is able to detect both an individual analyte and mixtures of analytes. Preferably, the present invention includes a plurality of reactants that may simultaneously detect many kinds of analytes rapidly. In addition, the present invention may be either reusable, assuming the reactants can be returned to their original state after use, or replaceable, if the relevant chemistry is not reversible or if dictated by reasons of hygiene or other issues of convenience and/or expense.

Figures 1 and 2 illustrate embodiments of a system of the present invention for detecting analytes in an air fluid sample. Such a system can be used to detect gas-born analytes, including, but not limited to, metabolic byproducts in bodily structures such as the esophagus, the gastrointestinal tract or the abdominal cavity.

As shown in Figures 1 and 2, the system 100 includes a sensor structure 101, a physical assembly 102 for proximally placing the sensor in a region of interest, and drive and interface electronics 103. The drive and interface

electronics may be connected either indirectly (Figure 1) or directly (Figure 2) to the physical assembly 102. In a preferred embodiment, the system 100 includes video pick-up assembly 104. Figures 3 and 4 illustrate embodiments of a system 110 of the present invention for detecting analytes in a liquid fluid sample. Such a system may be utilized to detect liquid-born analytes, including, but not limited to, detecting cardiac or tumor markers within biological serum; detecting different types of plaque within cardiac arterial walls; detecting electrolytes, ketones, blood glucose, and blood gas parameters in blood samples; detecting bacteria in a sample, such as a fluid sample in a gastric ulcer or lungs; detecting bacteria, pesticides, hormones, antibiotic and the like in wash from fruits, grains, or other products, or from liquid food products, such as, for example, milk, juices, canned soups and breakfast drinks; for environmental monitoring, including testing runoff water, ponds, lakes, rivers, wells, mining operations and testing toxins on a farm, in both soil and water; and for monitoring plants.

As shown in Figures 3 and 4, the system 110 includes a sensor structure 111, a detector 112, an illumination source 113, a physical assembly 114 for proximally placing the sensor in a region of interest, and drive and interface electronics 115. The sensor 111, the detector 112 and the illumination source 113 are all connected to the physical assembly 114 in some fashion. For example, either the sensor 111, the detector 112 and/or the illumination source

113 may be formed in a common structure with the physical assembly 114, such as, for example, in the same mold or die. In addition, either the sensor 111, the detector 112 and/or the illumination source 113 may be attached to the physical assembly 114 through any number of attachment mechanisms, including, but not limited to, the use of adhesives or fastening devices.

The drive and interface electronics 115 are connected either indirectly (Figure 3) or directly (Figure 4) to the physical assembly 114. In this embodiment, the detector 112 is positioned below the sensor structure 111 to allow for data acquisition. In a preferred embodiment, the system 110 includes video pick-up assembly 117. In another preferred embodiment, for fluorescent measurements, the system 110 includes a filter assembly 116 that removes the excitation wavelength.

Although any number of types of sensors may be utilized for the sampling of the fluid of interest, a preferred sensor structure is based on utilizing a sensor including at least one reactant. In particular, after the reactant interacts with the analyte of interest in the fluid sample, the reactant experiences spectroscopic changes that may be detected using a suitable detector. This preferred sensor structure is discussed in detail in the following U.S. Applications for Patent: Ser. Nos. 60/093,111, 09/354,882, 60/144,436, 60/144,435, 60/144,126 and PCT/US99/16162, all filed on 16 July 1999; Ser. No. 09/287,248 filed 7 April

1999; Ser. Nos. 60/179,369, 60/179,424, 60/179,294, 60/179,380, 60/179,380, 60/179,292 and 60/179,293, all filed on 31 January 2000; the foregoing all owned by the University of Texas and subject to license by Applicants; and Ser. No. 60/133,230, filed 7 May 1999, jointly owned by Applicants and the University of Texas, each of which is herein incorporated by reference in its entirely.

In one embodiment, the reactant is a plurality of chemically sensitive structures that produce a detectable signal in the presence of an analyte. Preferably, the reactants produce colorimetric or fluorescence signals upon exposure to an analyte. In a preferred embodiment, the sensor structure includes an array of such reactants.

Examples of the reactants include, but are not limited to, functionalized polymeric beads (e.g., polystyrene-polyethylene-glycol (PS-PEG) resin beads), gels, metal oxides particles (e.g., silicon dioxide [SiO_2] or aluminum oxides [Al_2O_3]), thin polymer films, metal quantum particles (e.g., silver, gold, platinum, etc.), semiconductor quantum particles (e.g., Si, Ge, GaAs, etc.), agarose, ceramics, glass, or any combination of the foregoing. The particular reactant selected is dependent on its specific wettability characteristics, porosity, and reflectivity. In this manner, the reactant selected will be ideally suited to detect the particular analyte desired to be detected.

In one embodiment, the reactant is a sensing particle, such as, for

example, PS-PEG resin beads. The choice of a PS-PEG matrix is based on its wettability by aqueous solutions and the availability of well developed literature methods for its derivatization as described in the experimental section.

In one embodiment, the reactant is a receptor molecule synthesized, adsorbed onto, attached to, or intercalated into a sensing particle. The particular receptor molecule chosen depends on which analyte one wants to detect. Examples of chemically active receptor molecules that may be formed on the surface of the sensing particle include a wide variety of molecules, including, but not limited to, signaling proteins (e.g., antigens, antibodies, and enzymes), chemically sensitive dyes, nucleic acid strands, nucleotide sequences, biological binding agents (e.g., avidin/biotin, lectins, and chelators), organic/inorganic metabolites, and functional group receptors.

In one embodiment, the sensing particles have diameters in the range of 1 - 500 microns and may actually change size (e.g., swell or shrink) when exposed to analytes in the fluid sample. For example, a fluid sample that includes a large amount of lipid material may cause non-polar particles to change in volume when the particles are exposed to such a fluid.

As discussed above, the sensor includes a substrate having a plurality of spatially distinct locations, with at least one of the plurality of spatially distinct locations having a reactant. In one embodiment, as shown in Figures 5 and 6,

the substrate is a support member 120 configured to include the reactants. The support member 120 is preferably used to localize the reactants, as well as to serve as a microenvironment in which chemical assays may be performed. The support member 120 is preferably made of any material capable of supporting the reactants, while allowing the passage of the appropriate wavelength of light. Preferably, the support member 120 is also made of a material substantially impervious to the fluid sample of interest. The support member 120 may be made of a variety of materials, including, but not limited to, silicon, plastics, glass, metals, resins, polymers, fiber composites, metals, metal alloys, ceramics or any combination of the foregoing.

As shown in Figures 5 and 6, the support member 120 preferably includes a plurality of cavities 130. In one embodiment, the cavities 130 are formed such that at least one particle 124 is substantially contained within a cavity 130. In an alternative embodiment, a plurality of particles are contained within a single cavity (not shown). A detailed description of the formation of cavities in a support member is discussed, for example, in U.S. Application Serial Nos. 90/287,248, filed April 7, 1999; 60/144,436, filed July 16, 1999; and 60/179,369, field January 31, 2000; each of which is subject to joint license by Applicants, and are herein incorporated by reference thereto.

As stated above, in addition to the sensor structure 111, the system 110

includes a detector 112 and an illumination source 113. As shown in Figure 5, light comprising a portion of the electromagnetic spectrum, which originates from the illumination source 113, preferably passes through the sensor structure or array 111 and is modulated by the reactants of the sensor structure, before impinging on the proximally spaced detector 112. For fluorescent based systems, the light source 113 is a wavelength tailored and preferably filtered source, such as a light emitting diode (LED), whose frequency and wavelength output is chosen to compliment the excitation band of the chosen sensing fluorophore particle For example, in one embodiment, a high intensity blue LED is appropriate complement to fluorescein. For colorimetric (e.g., absorbance) based systems, the light source 113 is preferably a "white" light. In one embodiment, the light is delivered proximate to the sensor through a fiber optic assembly (not shown).

Monitoring the spectroscopic changes to the reactants is preferably accomplished by use of the detector 112 itself and may be assisted by a microprocessor (such as microprocessor 140 shown in Figure 6) (or other optical or electronic processing system components) coupled, directly or indirectly, to the detector 112.

The detector 112 measures changes in the optical characteristics which occur upon the interaction of the reactants with the analytes. A wide range of

optical detectors, including, but not limited to, charged coupled detectors (CCD), ultraviolet detectors or fluorescent detectors, may be used. For fluorescence measurements, as shown in Figure 6, a band limiting or band pass optical filter 116 may be placed between the support member and detector 112 to remove the excitation wavelength.

The system 110 of the present invention includes a sensor structure 111 with an integral detector 112 to allow the detection of any analyte to occur within the region of interest. The physical assembly 114 is used to place the sensor structure 111 and the detector 112 within the region of interest. In human or animal applications, to place the sensor structure 111 and the detector 112 in the region of interest, the physical assembly 114 is configured to be inserted through an opening in the biological system, such as, for example, an existing orifice or incision, to locate the physical assembly 114 (with sensor and detector) proximate to the region of interest. In one embodiment, the sensor structure 111 and the detector 112 are integrated into a typical catheter that is used to provide central venous access for hydration.

Because the present invention may be used in in vivo or in vitro applications, there are engineering constraints that must be observed in order to make the present invention compatible with the body. In particular, the physical assembly 114 must be of a shape and size that permits it to be delivered

proximate to the region of interest with minimal invasive effects, typically requiring the overall diameter to be 15 mm or less. In addition, the physical assembly 114 should be smooth, must be constructed of bio-compatible materials, and must operate at safe energy levels and temperatures. In one embodiment, the physical assembly 114 has the shape of a probe. In another embodiment, the physical assembly 114 is spheroidal in shape.

In addition to the sensor structure 111, detector 112, illumination source 113, and the physical assembly 114, the system 110 includes drive and interface electronics 115. In one embodiment, as shown in Figure 3, the drive and interface electronics 115 are indirectly connected to the physical assembly 114. The indirect connection of the drive and interface electronics 115 with the physical assembly may include, but is not limited to, utilizing wires. One of skill in the art can also readily recognize that alternative indirection connections include, but are not limited to, utilizing induction methods, such as using radio waves, microwaves, or infrared sensing. In an alternative embodiment, as shown in Figure 4, the drive and interface electronics 115 are directly connected to the physical assembly 114. In one embodiment, the drive and interface electronics 115 are attached to one end of the physical assembly 114.

The drive and interface electronics 115 include, but are not limited to, well known electrical components such as, for example, a microprocessor to acquire

data and to manipulate the data, memory chips to store the data, timing circuitry, calibration circuitry, communication interface circuitry, provisions to handle applicable device triggering and the like. These types of electrical components are well known in the electrical arts, and a detailed discussion of each is beyond the scope of this invention.

In a preferred embodiment, the present invention includes means for retrieving optical information from within the body of interest. In particular, the present invention preferably includes optical video technology that acquires and permits the display of imagery from within the body of interest that can be used to visualize and inspect the areas of interest, as well as performing localized chemical/biochemical assays. In particular, as shown in Figures 1 through 4, video pick-up assembly 104 or 117 allows one to view the area where the physical assembly 102 or 114 is being positioned. The video pick-up assembly 104 or 117 is preferably a video monitoring system to allow one to view the region of interest as the physical assembly 102 or 114 is being inserted to the region of interest.

Optical Systems

Presently, optical sensors may be based upon colorimetric or fluorescent based systems. For either system, the sensor is generally made up of reactants

which experience spectroscopic changes after interacting with an analyte of interest. In addition, for either system, a detector is utilized to detect the spectroscopic changes experienced by the reactants.

Colorimetric Based Systems

Evaluation of the changes in optical properties of the various reactants in a colorimetric based system may be accomplished in the following fashion. Light is delivered to the sensor structure and the detector records the colorimetric changes in the light that passes through the reactants. A commercial-grade 24-bit (8 bit x 3 color planes) video camera (not shown) is preferably used to detect the reactant changes, providing the ability to acquire images at a rate of approximately 30 times per second. This speed is valuable in the initial kinetic characterization of reactants, such as, for example, micro-sphere sensors. Although not discussed at length here, evaluation of the transient data could provide an additional means to identify the chemical composition of these samples. It should be emphasized that the rapid acquisition of time dependent colorimetric data provides a particularly powerful analytical capacity when these capabilities are combined with modem pattern recognition algorithms.

For colorimetric based systems, the spectral information is acquired from the detector in a format where the white light has been simplified, for instance, by

assigning relative intensity values to three key wavelength regions, namely red-green-blue (RGB). This interpretation has been adopted from standard color wheel analysis protocols and represents one Commission Internationale de L'Eclairage (CIE) standard color space that can be used for colorimetric processing. The linear intensity levels recorded with the detector are normalized against an averaged background light level transmitted through particles which are underivatized. The level of the averaged background light level through underivatized particles is taken as 100% transmittance. In a reactant particle-based system, it should be noted, however, that transmission intensities recorded through a particle are typically 20% less than those obtained in the absence of the particle, as expected since unavoidable light scattering occurs. For this reason, underivatized particles in the cavities are used as reference cells instead of empty cavities.

Absorptance ($A_{R,G,B}$) values reported for each of the three RGB hues are determined from the following equation:

$$A_{R,G,B} = 1 - \left[\frac{T_{R,G,B}}{T_o}\right]$$

where $T_{R,G,B}$ is defined at the transmitted intensity through a particle in a particular environment for each of the selected hues, and T_o is the average background intensity through the underivatized particles. The coherent appearance of color

through the center of the particle indicates that the analyte is incorporated uniformly throughout the particle. Collectively, these results demonstrate that aqueous analytes can penetrate into the polymer particles, that the particles are suitable media for optical measurements, and that the detector can be used to quantify the colorimetric changes. Other systems may be used wherein the light source and the sensor operating frequency and wavelength include the receptor activation range. The term "receptor activation range" is thus understood herein to refer to that portion of the electromagnetic spectrum in which the receptor absorbs or interacts with electromagnetic radiation.

Fluorescent Based Systems

Fluorescent based diagnostic assays operate under the same general principles, although they differ in the optical excitation and signal output methodology. In a particle-based reactant system, the actual receptor molecule design and attachment to the sensing particle is identical to colorimetric techniques. In the fluorescent mode, the reactant emits a fluorescent rather than a colorimetric signal. This is accomplished through the conjugation of chemically sensitive fluorophores to both the particle substrate and the custom designed receptor compounds. These fluorophores, responding to local binding events in and around the particle, emit a unique fluorescent signature. However, this

requires excitation via a specific wavelength of light, either through the use of optical filters or specifically tuned excitation sources such as light emitting diodes (LED). In particular instances, fluorescent signaling schemes are employed to amplify signal output from otherwise indetectable analytes. Signal detection is still accomplished using an optical detector such as, for example, a CCD detector. This information is then processed and both qualitative and quantitative values are achieved using correlations with the intensity of the specific wavelength of light emitted from the reactants. In this scenario, signal analysis is simplified due in part to the narrow spectral signal input.

Example of Optical Detection Based on *o*-cresolphthalein Complexone Derivatized Micro-spheres Utilizing Colorimetric Based System

The following is an illustrative example showing how a particle reactant can acquire information related to the local chemistry of fluids based on a colorimetric based system. The information is obtained from optical photomicrographs that were taken of *o*-cresolphthalein complexone derivatized particles or micro-spheres. Upon the exposure of the particles to aqueous solutions containing no Ca^{+2} and 0.1 M Ca^{+2}, these micro-spheres change color in a dramatic manner. Indeed, the micro-spheres are virtually transparent across the entire visible region when no Ca^{+2} is present in the solution. In contrast, the same micro-sphere exposed to 0.1 M Ca^{+2} displays a markedly different physical appearance as

indicated by purple hue. The *UV-vis* absorbance spectrum reveals the absorption band is centered at = 550 nm which approximately matches the solution absorbance spectrum of *o*-cresolphthalein complexone responding to Ca^{+2}.

The sensor structure of the electronic tongue sensor as described herein has the capacity to simultaneously analyze the chemical composition of complex mixtures of analytes. In particular, the composite changes to different analytes were analyzed for four different environments including no analyte, 0.1 M Ca^{+2}, 0.1 M Ce^{+3}, a mixture of 0.1 M Ca^{+2} and 0.1 M Ce^{+3} for four different micro-spheres. The four different micro-spheres were tagged with: (1) underivatized, (2) *o*-cresolphthalein complexone, (3) alizarin complexone, and (4) fluorescein. For each of the environments on each of the sensors, five representative pH's (3,5,7,9, and 11), all buffered by a mixture of 0.04 M phosphate, 0.04 M acetate, and 0.04 M borate, were evaluated.

Quantification of colorimetric changes using the RGB analysis were compiled for each of four chemically functionalized micro-spheres. This protocol conforms to the capabilities afforded by commercially available CCD-based detectors. With the use of the CCD detector, three channels of data are available for each micro-sphere as a function of time. In fact, since the micro-sphere is significantly larger than the associated CCD pixels, redundant information is available allowing for a signal averaging to be made for each micro-sphere.

For the underivatized micro-sphere, little light attenuation is observed for all of the studied cases. A small deviation in the signals recorded for this micro-sphere suggests minor alterations in the light scattering changes in the absorbance properties or differences in the alignment occurred. In any event, the differences are relatively small when compared to the other micro-sphere structures. In fact, each type of micro-sphere displays spectral features and chemical selectivity vastly different from the other ones. For example, the *o*-cresolphthalien micro-spheres display significant color attenuation only in the two cases at pH = 11 with Ca^{+2} alone and Ca^{+2}/Ce^{+3} mixtures. In particular, in these situations, most significant attenuation occurs in green wavelengths, along with modest color attenuation in red wavelengths. In all other chemical environments, the color attenuation is less significant.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A method of detecting an analyte in a fluid sample comprising the step of analyzing the fluid sample integral to a region of interest with a proximally located sensor with an integral detector.

2. The method of claim 1 wherein the detector is integral with the sensor by forming the sensor and the detector in a common structure.

3. The method of claim 1 wherein the detector is integral with the sensor by attaching the detector to the sensor.

4. The method of claim 1 wherein the detector is integral with the sensor through an optical path from the sensor to the detector.

5. The method of claim 4 wherein the optical path comprises an optical fiber.

6. The method of claim 1 wherein the analyzing step comprises the steps of: providing, for the sensor, a substrate with a plurality of spatially distinct locations, at least one of the plurality of spatially distinct locations having a reactant;

interacting the fluid sample with the reactant,

transmitting a light source comprising one or more portions of the electromagnetic spectrum to the reactant, and

monitoring the reactant to detect the analyte.

7. The method of claim 6 further comprising the step of providing a plurality of reactants, wherein each reactant is placed at at least one of the plurality of spatially distinct locations.

8. The method of claim 6 further comprising the step of providing a plurality of reactants, wherein more than one reactant is placed at at least one of the plurality of spatially distinct locations.

9. The method of claim 6 wherein the substrate defines a plurality of cavities, wherein at least one cavity is placed at at least one of the plurality of spatially distinct locations.

10. The method of claim 9 further comprising a plurality of reactants, wherein each reactant is placed in at least one of the plurality of cavities.

11. The method of claim 9 further comprising providing a plurality of reactants, wherein more than one reactant is placed in at least one of the plurality of cavities.

12. The method of claim 6 wherein the reactant is selected from the group consisting of a sensing particle, a receptor molecule attached to a sensing particle, a gel or a reactive material.

13. The method of claim 12 wherein the sensing particle is selected from the group consisting of functionalized polymeric beads, metal oxide particles, thin polymer films, metal quantum particles, semiconductor quantum particles,

agarose, ceramic, glass, or any combination of the foregoing.

14. The method of claim 12 wherein the receptor molecule is selected from the group consisting of signaling proteins, chemically sensitive dyes, nucleic acid strands, nucleotide sequences, biological binding agents, antibodies, antigens, organic metabolites, inorganic metabolites, functional group receptors, or any combination of the foregoing.

15 The method of claim 6 wherein the substrate is comprised of glass, plastic, silicon, resin, polymer, fiber composite, metal, metal alloy, ceramic, or any combination of the foregoing.

16. The method of claim 6 wherein the reactant is a fluorescent based reactant, and the light source is selected such that the frequency and wavelength of the light compliments the excitation band of the selected fluorescent based reactant.

17. The method of claim 6 wherein the reactant is a colorimetric based reactant, and the light source is a portion of the electromagnetic spectrum.

18. The method of claim 17 wherein the light source is a white light.

19. The method of claim 6 wherein the step of monitoring the reactant comprises monitoring the spectroscopic changes of the reactant by the detector as the fluid sample engages the reactant, wherein the spectroscopic changes are caused by the analyte present in the fluid sample interacting with the reactant.

20. A method of analyzing a fluid sample containing one or more analytes in a biological system comprising the steps of:

providing a physical assembly, the physical assembly including a sensor, a detector and an illumination source, the sensor including a substrate with a plurality of spatially distinct locations, at least one of the plurality of spatially distinct locations having a reactant;

introducing the physical assembly proximal to a region of interest where a fluid sample is present;

interacting a fluid sample with the reactant;

transmitting a light source comprising a portion of the electromagnetic spectrum to the reactant; and

monitoring, utilizing the detector, the reactant to detect an analyte while the detector is proximally located to the region where the fluid sample is present.

21. The method of claim 20 further comprising the step of providing means for retrieving optical information from within the biological system.

22. The method of claim 21 wherein the means for retrieving optical information comprises a video monitor.

23. The method of claim 20 further comprising the step of providing drive and interface electronics connected to the physical assembly.

24. The method of claim 23 wherein the drive and interface electronics are directly connected to the physical assembly.

25. The method of claim 23 wherein the drive and interface electronics are indirectly connected to the physical assembly.

26. The method of claim 20 wherein said physical assembly is a probe.

27. The method of claim 20 wherein said physical assembly is spheroidal in shape.

28. The method of claim 20 wherein the step of monitoring the reactant comprises monitoring the spectroscopic changes of the reactant by the detector as the fluid sample engages the reactant, wherein the spectroscopic changes are caused by the analyte present in the fluid sample interacting with the reactant.

29. A method of detecting an analyte in a fluid sample within a biological system comprising the step of analyzing the fluid sample while inside the biological system with a sensor and a detector which is integral with the sensor, wherein the sensor and the integral detector are inserted into the biological system to be proximally located to the fluid sample in a region of interest, and the detector detects the analyte present in the fluid sample while the detector is proximally located to the fluid sample in the region of interest.

30. The method of claim 29 wherein the biological system is a

mammalian body, such as a human, either living or dead.

31. The method of claim 30 wherein the sensor and the detector are integrated with a catheter to introduce the sensor and the detector to the region of interest.

32. The method of claim 31 wherein the biological system is a mammalian body, such as a human, either living or dead.

33. A device for detecting an analyte in a fluid sample integral to a region of interest comprising:

a physical assembly, the physical assembly including a sensor, a detector which is integral to the sensor, and an illumination source; and

the sensor comprised of a substrate having a plurality of spatially distinct locations, at least one of the spatially distinct locations having a reactant;

wherein the physical assembly introduces the detector proximate to a fluid sample integral to a region of interest to allow the detector to monitor the reactant while proximally located to the fluid sample.

34. The device of claim 33 wherein the detector is integral with the sensor by forming the sensor and the detector in a common structure.

35. The device of claim 33 wherein the detector is integral with the sensor by attaching the detector to the sensor.

36. The device of claim 33 wherein the detector is integral with the

sensor through an optical path from the sensor to the detector.

37. The device of claim 36 wherein the optical path comprises an optical fiber.

38. The device of claim 33 further comprising a plurality of reactants, wherein each reactant is placed at at least one of the plurality of spatially distinct locations.

39. The device of claim 33 further comprising a plurality of reactants, wherein more than one reactant is placed at at least one of the plurality of spatially distinct locations.

40. The device of claim 33 wherein the substrate defines a plurality of cavities, wherein at least one cavity is placed at at least one of the plurality of spatially distinct locations.

41. The device of claim 40 further comprising a plurality of reactants, wherein each reactant is placed in at least one of the plurality of cavities.

42. The device of claim 40 further comprising a plurality of reactants, wherein more than one reactant is placed in at least one of the plurality of cavities.

43. The device of claim 33 wherein the reactant is selected from the group consisting of a sensing particle, a receptor molecule attached to a sensing particle, a gel or a reactive material.

44. The device of claim 43 wherein the sensing particle is selected from the group consisting of functionalized polymeric beads, metal oxide particles, thin polymer films, metal quantum particles, semiconductor quantum particles, agarose, ceramic, glass, or any combination of the foregoing.

45. The device of claim 43 wherein the receptor molecule is selected from the group consisting of signaling proteins, chemically sensitive dyes, nucleic acid strands, nucleotide sequences, biological binding agents, antibodies, antigens, organic metabolites, inorganic metabolites, functional group receptors, or any combination of the foregoing.

46. The device of claim 33 wherein the substrate is comprised of glass, plastic, silicon, resin, polymer, fiber composite, metal, metal alloy, ceramic, or any combination of the foregoing.

47. The device of claim 33 wherein the reactant is a fluorescent based reactant, and the light source is selected such that the frequency and wavelength of the light compliments the excitation band of the selected fluorescent based reactant.

48. The device of claim 33 wherein the reactant is a colorimetric based reactant, and the light source is a portion of the electromagnetic spectrum.

49. The device of claim 48 wherein the light source is a white light.

50. The device of claim 33 further comprising means for retrieving

optical information from within the region of interest.

51. The device of claim 50 wherein the means for retrieving optical information comprises a video monitor.

52. The device of claim 33 wherein said physical assembly is a probe.

53. The device of claim 33 wherein said physical assembly is spheroidal in shape.

54. The device of claim 33 wherein the sensor and the detector are integrated with a catheter to introduce the sensor and the detector to the region of interest.

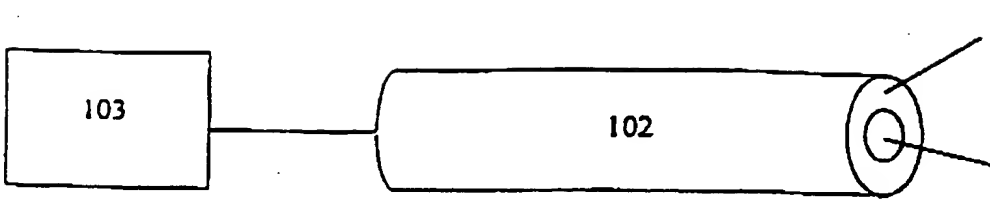


FIGURE 1

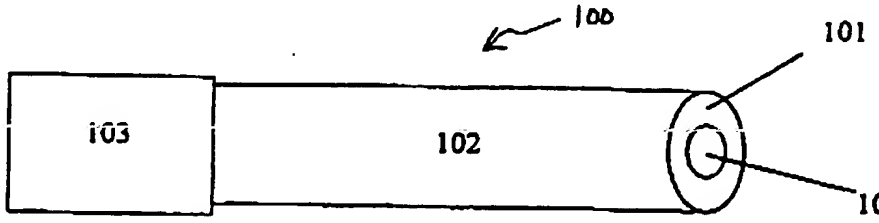


FIGURE 2

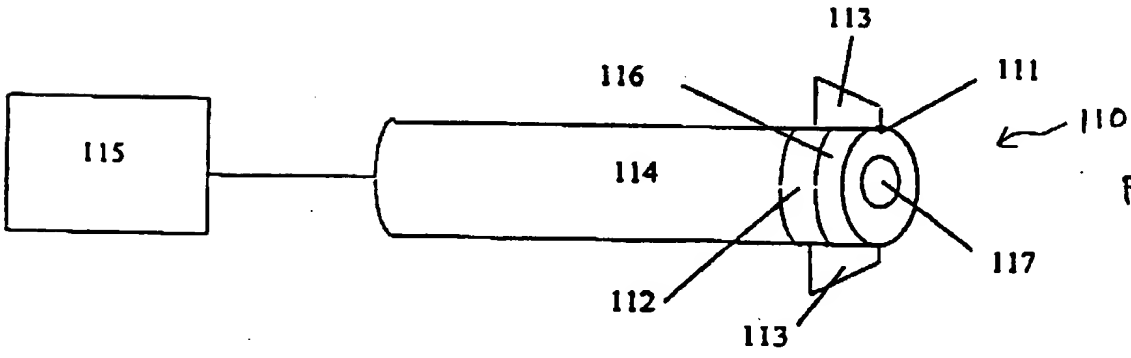


FIGURE 3

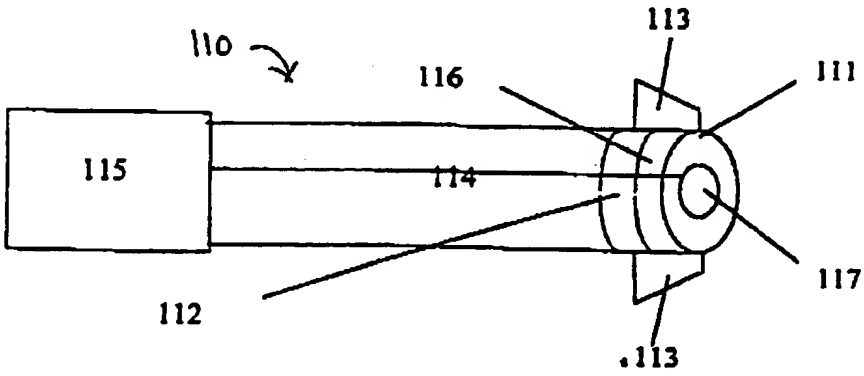


FIGURE 4

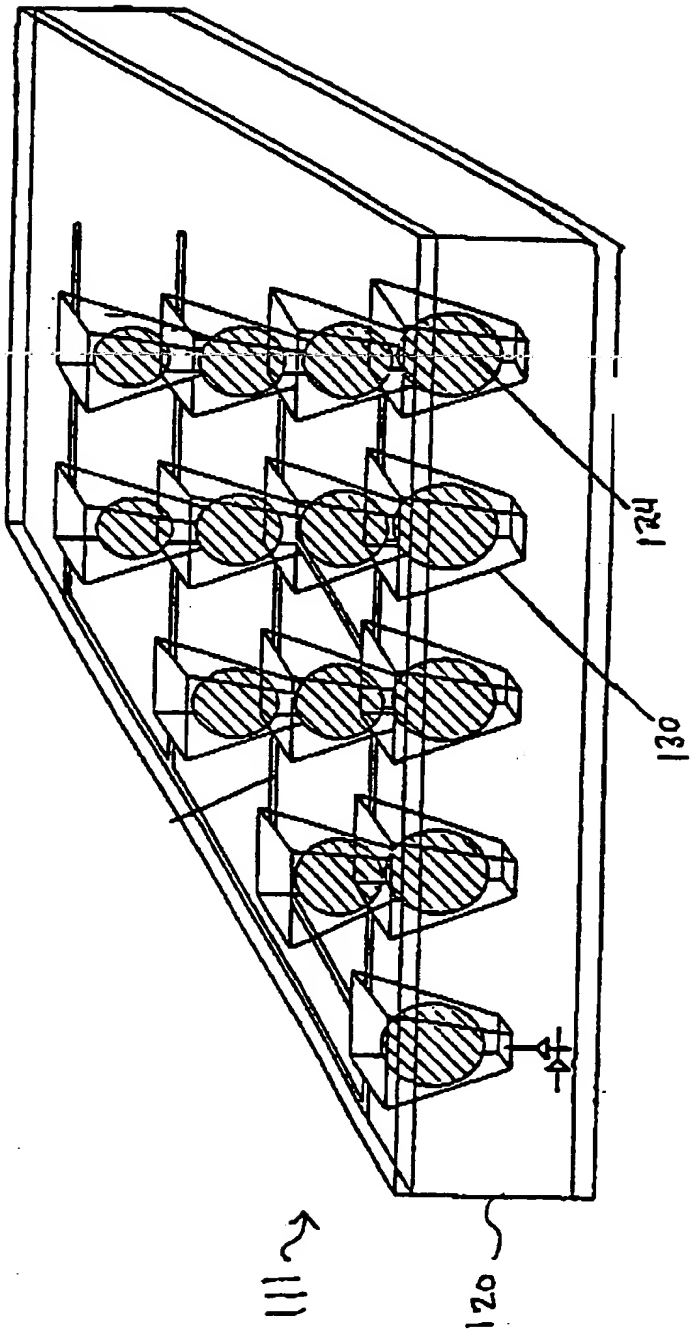


FIG. 5

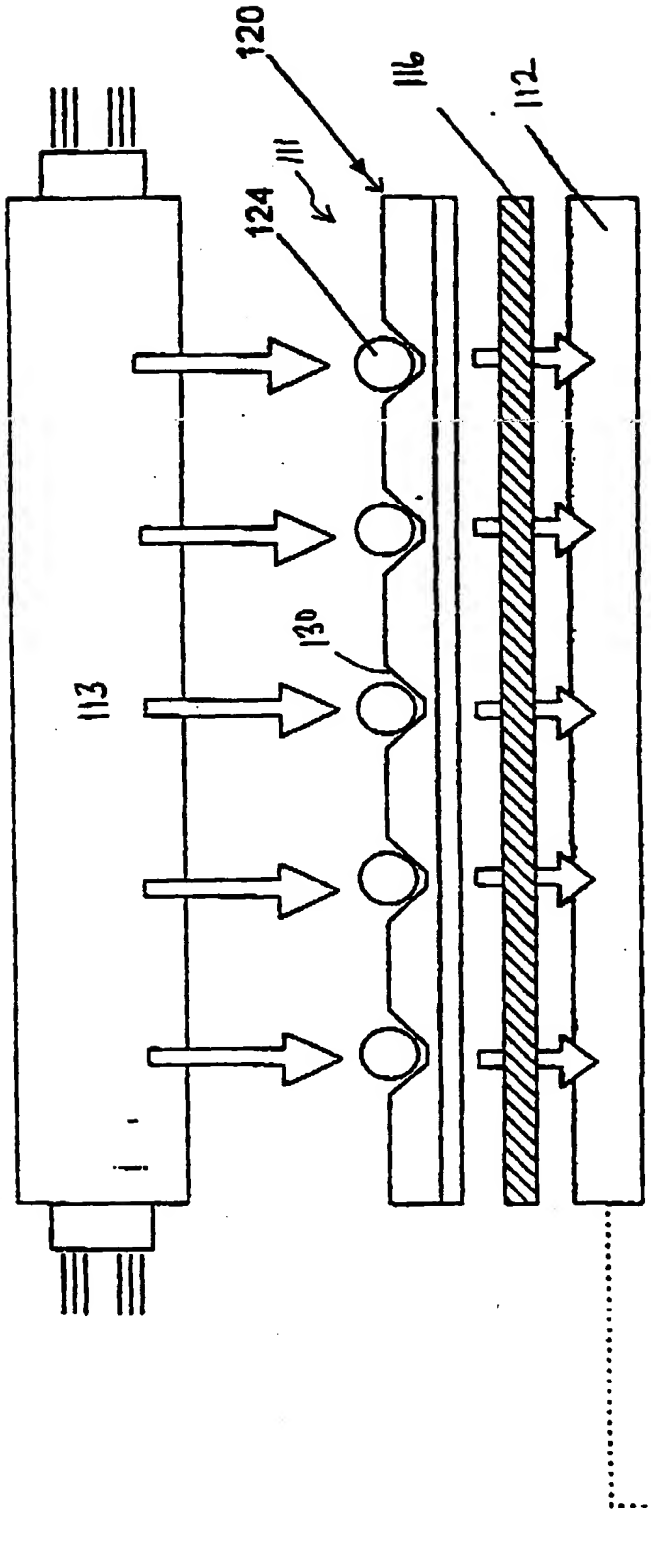


FIG. 6

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 00/12409

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64 G01N21/77

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 29736 A (GENOMETRIX INC) 9 July 1998 (1998-07-09) claim 1; figures 1,9,10 ---	1-54
A	WO 98 53300 A (BRENNER SYDNEY ;LYNX THERAPEUTICS INC (US); BRIDGHAM JOHN (US); CO) 26 November 1998 (1998-11-26) figure 1A ---	1-54
X	US 5 109 849 A (GOODMAN DAVID E ET AL) 5 May 1992 (1992-05-05) figures 4,7A-7B ---	1-54
X	US 5 244 636 A (WALT DAVID R ET AL) 14 September 1993 (1993-09-14) figure 4 ---	1-54

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2000	27/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mason, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/12409

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 233 668 A (YOKOMORI YASUHIKO ET AL) 3 August 1993 (1993-08-03) figure 1	1-54
A	WO 98 25701 A (UNIV CALIFORNIA) 18 June 1998 (1998-06-18) figure 3	1-54
A	US 5 800 341 A (MCKENNA MICHAEL A ET AL) 1 September 1998 (1998-09-01) figures 1,4,6	1-54

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.nformation on patent family members

Inte onal Application No
PCT/US 00/12409

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9829736 A	09-07-1998	AU 6646398 A	31-07-1998
		EP 0990142 A	05-04-2000
WO 9853300 A	26-11-1998	AU 7715598 A	11-12-1998
		EP 0985142 A	15-03-2000
		US 6013445 A	11-01-2000
US 5109849 A	05-05-1992	US 4938218 A	03-07-1990
		AT 151248 T	15-04-1997
		AU 645962 B	03-02-1994
		AU 4523889 A	14-05-1990
		CA 1334210 A	31-01-1995
		DE 68927958 D	15-05-1997
		DE 68927958 T	22-01-1998
		EP 0440741 A	14-08-1991
		JP 2740561 B	15-04-1998
		JP 4501520 T	19-03-1992
		WO 9004352 A	03-05-1990
		US 5099842 A	31-03-1992
		EP 0135840 A	03-04-1985
		JP 60090535 A	21-05-1985
US 5244636 A	14-09-1993	US 5244813 A	14-09-1993
		US 5320814 A	14-06-1994
		US 5250264 A	05-10-1993
US 5233668 A	03-08-1993	JP 2886947 B	26-04-1999
		JP 4029036 A	31-01-1992
		DE 4117024 A	28-11-1991
WO 9825701 A	18-06-1998	AU 5799198 A	03-07-1998
		EP 0948408 A	13-10-1999
US 5800341 A	01-09-1998	US 5547455 A	20-08-1996
		CA 2186881 A	12-10-1995
		EP 0754004 A	22-01-1997
		WO 9526674 A	12-10-1995